

Intestinal Transport: Studies with Isolated Epithelial Cells

by George A. Kimmich*

Isolated intestinal epithelial cells have been extremely useful for characterizing the nature of intestinal absorption processes and for providing insight into the energetics of Na^+ -dependent transport systems. This report describes a number of experimental approaches which have been used for investigating the specific epithelial transport systems involved in sugar absorption, but provides information which ultimately should prove useful for characterizing a number of different intestinal transport events. Similar experiments should also prove useful for exploring the effect of environmental agents on the function of intestinal tissue.

In the case of sugars, net absorption is accomplished via a mucosal, Na^+ -dependent concentrative transport system acting in sequence with a passive serosal system which does not require Na^+ . The serosal system limits the full gradient-forming capability of the mucosal system. Agents such as phloretin or cytochalasin B which inhibit serosal transport allow the cells to establish sugar gradients as high as 70 fold in contrast to 10-15 fold gradients observed for control cells. Seventy-fold sugar gradients cannot be explained in terms of the energy available in the electrochemical potential for Na^+ if the Na^+ :sugar coupling stoichiometry is 1:1 as commonly assumed. New information indicates that the true Na^+ :sugar stoichiometry is in fact 2:1. Flow of two Na^+ ions per sugar molecule down the transmembrane electrochemical potential for Na^+ provides more than sufficient energy to account for observed 70 fold sugar gradients. If flow of sugar by other routes could be completely inhibited, theoretical sugar gradients as high as 400 could be achieved assuming that the cells maintain a membrane potential of -36 mV as measured for intact tissue.

Introduction

The primary physiological function of small intestinal tissue is to extract selectively a variety of nutrients from the complex mixture of dietary constituents presented to it and transfer them to the circulatory system from which they then have access to every organ system. With the exception of oxygen, none of the solutes essential to the survival of every living cell can be delivered to those cells without first having been absorbed by the intestine. In a very real sense, then, proper functional capability for each tissue is dependent upon normal function of the intestinal nutrient transport systems. For many nutrients, the intestine has capability for concentrative transfer of material against relatively large gradients of chemical potential. In these instances, an expenditure of metabolic energy is mandatory to establish and maintain the concentration gradients

observed between intestinal lumen and the lamina propria region of the intestinal villi. This energy expenditure helps insure essentially complete removal of nutrient solutes from the diet for use by the body. A complete consideration of intestinal absorption events must therefore take account of transport energetics as well as the function of the specific transport proteins.

It is important to recognize that intestinal absorption is the summation of sequentially acting transport systems located at the brush border and serosal boundaries of the columnar epithelial cells which line the luminal-facing surface of the villi. Solute which are transferred across both membranes have immediate access to the circulatory system without the necessity for participation of other transport systems. For this reason, intestinal absorption is primarily a function of the activity of the intestinal epithelium, and the transport characteristics of the intact tissue can be ascertained in fundamental terms from a consideration of the capability of the cells which comprise this epithelium. By the same token, toxic agents which interfere with the cellular trans-

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port capability will be candidates for exerting toxic effects on the absorptive capacity of the intact tissue.

In the following text, I hope to describe how use of a preparation of isolated intestinal epithelial cells has been of exceptional benefit in helping establish fundamental information related to the energetics of intestinal transport systems for sugars. In the course of describing use of this test system to explore a specific transport system, I believe the potential utility of the system for exploring absorption in a general sense will be exemplified, as well as the means by which the system can be used to evaluate the effects of toxic agents on intestinal absorption.

Methods

All of the data described here were obtained with suspensions of isolated intestinal epithelial cells obtained from chickens 5-6 weeks of age. The cells were prepared by the hyaluronidase-mechanical agitation method developed in our laboratory which has been described in detail in earlier reports (1, 2). The preparation yields a suspension of small clumps of cells which can be readily handled by micropipet and which can therefore be used for rapid sampling procedures important for the determination of unidirectional solute fluxes. Each sample reflects the average capability of cells in the suspension so that errors encountered with intact tissue preparations related to the necessity of sampling different sections of tissue for replicate samples are avoided. Transport capability is proportional to the amount of cellular protein used, which is therefore a convenient basis of comparison for data derived from different cell preparations.

The usual isolation and incubation medium consists of 80 mM NaCl, 55 mM Tris-Cl (pH 7.4), 3 mM K_2HPO_4 , 1 mM $MgCl_2$, 1 mM $CaCl_2$, and 1 mg/ml bovine serum albumin. Unless otherwise noted, 10 mM mannose, 2.5 mM glutamine, and 0.5 mM β -hydroxybutyrate were included as oxidizable substrates. Tris-Cl can be used to replace NaCl for experiments in which Na^+ must be avoided. Cells isolated in a Tris-Cl medium regain their full transport capability when Na^+ is added.

When the cells are chilled to 4°C, their membranes become impermeable to sugar. Uptake studies can therefore be performed by allowing an appropriate interval of ^{14}C -sugar uptake at 37°C, taking an aliquot of the suspension, and diluting that aliquot in a large volume of ice cold medium. The diluted sample can then be centrifuged and the supernatant discarded in order to separate the cells from the extracellular sugar. The large volume of diluent prevents significant extracellular trapping of sugar in the

pellet and the low temperature allows the cells to retain sugar previously accumulated. A wash with ice cold medium is sometimes necessary for complete removal of isotope "trapped" by the pellet. Intracellular ^{14}C -sugar can be released by adding 3% perchloric acid to the cell pellets. After centrifuging down the denatured protein an aliquot of the supernatant is counted in order to quantitate the amount of sugar accumulated during the incubation interval. 3-*O*-Methylglucose is used as the sugar of choice because it cannot be phosphorylated or otherwise metabolized by the cells.

Results and Discussion

In order to critically evaluate kinetic and other characteristics for any biological transport system, it is imperative that the test system can be utilized for monitoring unidirectional fluxes of the solute of interest. The degree to which the system meets this requirement is usually determined by procedures which assess the relationship between solute accumulated and time of incubation with solute. Linearity in this relationship indicates that a true

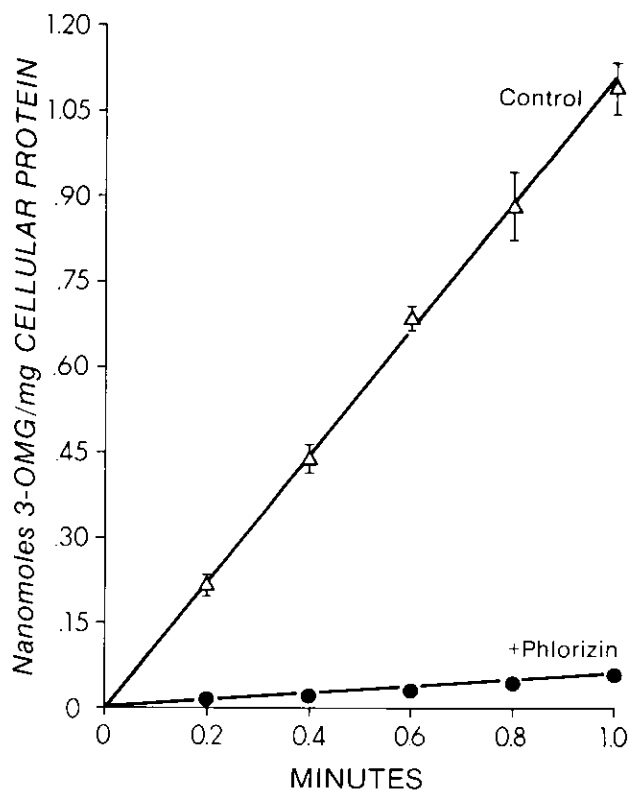


FIGURE 1. Effect of 200 μM phlorizin on unidirectional influx of 100 μM ^{14}C -3-OMG into isolated intestinal epithelial cells. The incubation medium contained 80 mM Na^+ . From Kimmich and Randles (6).

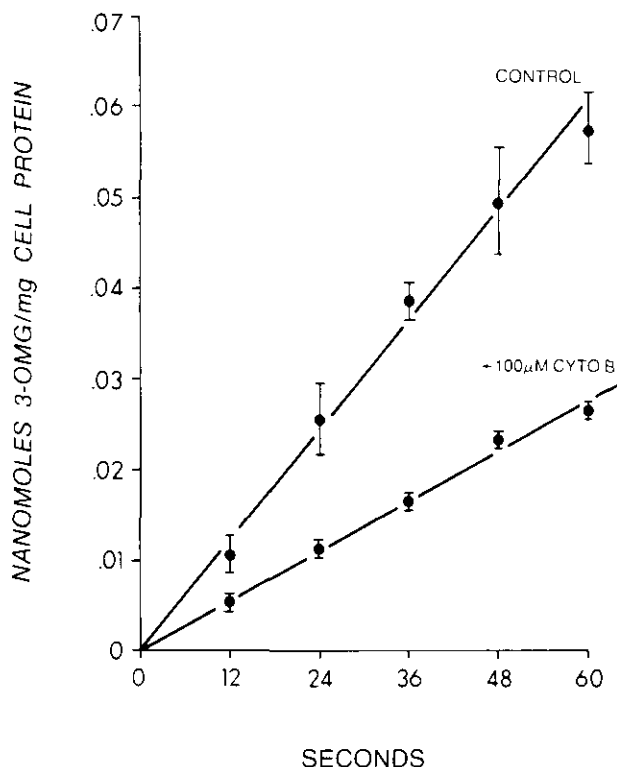


FIGURE 2. Effect of 100 μ M cytochalasin B on Na^+ -independent unidirectional influx of 100 μ M ^{14}C -3-OMG into isolated intestinal epithelial cells. The Na^+ -free incubation medium contained 80 mM Tris-Cl (pH 7.4) in place of NaCl.

unidirectional flux has been measured in a manner that is uncomplicated by solute backflux. Isolated chicken intestinal epithelial cells meet this requirement for a 1-min period of incubation with ^{14}C -3-OMG as shown in Figure 1. The data shown are for three separate experiments run with the same cell preparations and were obtained with an extracellular 3-OMG concentration of 100 μ M. Note that approximately 95% of the total sugar influx is sensitive to 200 μ M phlorizin, a plant glycoside which is known to inhibit influx of sugar across the brush border surface of intact tissue (3, 4). The same percentage of the total influx can be prevented by removing Na^+ from the incubation medium as described elsewhere (5). Because Na^+ dependence and phlorizin sensitivity are characteristic of sugar transport by only the brush border membrane of the intestinal epithelium (3, 4), the data in Figure 1 indicate that most of the sugar entry to the intact cell occurs via this route. On the other hand, a part of the phlorizin-insensitive flux of sugar can also be controlled. Figure 2 shows that in the absence of Na^+ about 60% of the total influx is sensitive to 100 μ M cytochalasin B (6). Higher concentrations of cytochalasin B have no greater effect. A number of

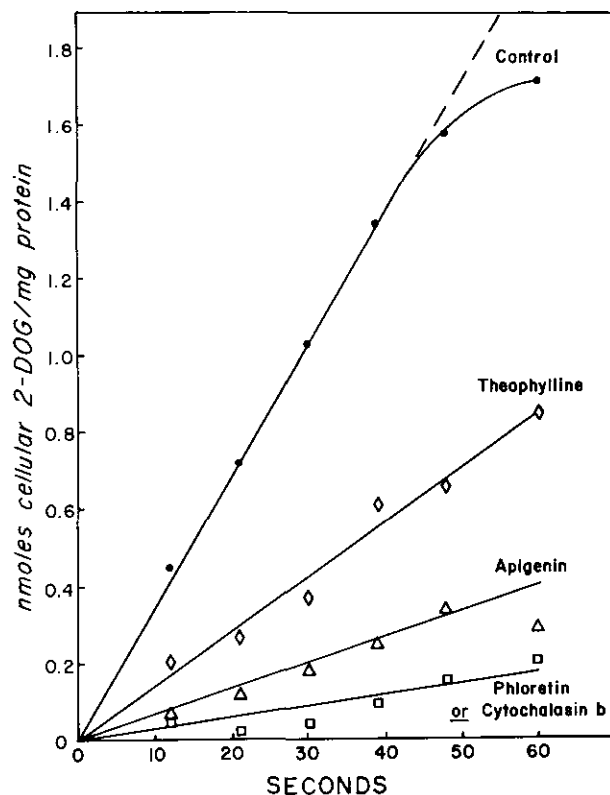


FIGURE 3. Effect of theophylline (5 mM), apigenin (200 μ M), phloretin (200 μ M), and cytochalasin B (500 μ M) on Na^+ -independent influx of 1 mM 2-deoxyglucose into isolated intestinal epithelial cells. 2-Deoxyglucose is a specific substrate for the Na^+ -independent sugar transport system and does not satisfy the Na^+ -dependent system. From Kimmich, Carter-Su, and Randles (9).

other agents can also be shown to inhibit the Na^+ -independent sugar influx including theophylline (7), various flavanoids (8), and phloretin (5, 7) (see Fig. 3). Of these agents, only phloretin is as potent in its action as cytochalasin B (6, 9). Under some conditions, phloretin is slightly more potent than cytochalasin, but it also causes a degree of metabolic inhibition which is not found with cytochalasin (7).

The small residual 3-OMG flux observed in the absence of Na^+ but with cytochalasin B present does not have any of the characteristics expected for a carrier-mediated event. It does not show any evidence for saturation by substrate; cannot be diminished by high concentrations of 3-OMG analogs; and is not inhibited by a variety of metabolic or transport inhibitors. For this reason, we believe it represents a non-mediated diffusional event.

Sugar influx into the isolated intestinal epithelial cells via the three routes defined above is represented schematically in Figure 4. Note that the phloretin (or cytochalasin B) sensitive system has been attributed to the lateral-serosal cell boundary.

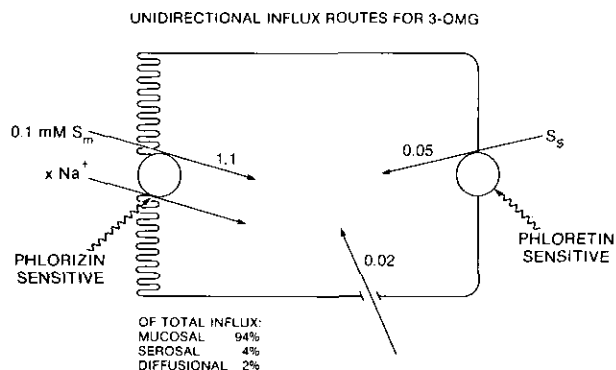


FIGURE 4. Schematic representation of unidirectional influx routes for $100 \mu M$ 3-OMG into isolated intestinal epithelial cells. Values given are in nmole/min-mg protein. The phlorizin-sensitive flux is coupled to a flux of Na^+ and occurs at the mucosal (brush border) boundary. The phloretin-sensitive flux is not dependent on Na^+ and is serosally localized. Precise localization of the diffusional flux is unknown. From Kimmich and Randles (6).

This is in accordance with data derived from isolated membrane vesicles which indicates a high degree of localization at that surface (10, 11). The diffusional entry route is shown as if it occurs at the lateral cell boundary although no specific information is available to demonstrate this choice. Recognize, however, that any passive flux route associated with the lateral-serosal boundary would serve to deliver sugar from the intestinal cell to the bloodstream during intervals when dietary sugar is available and concentrated within the cell by the Na^+ -dependent brush border transport system. Conversely, lateral-serosal passive systems can deliver nutrient molecules from the blood to the enterocyte during periods of fasting.

The numerical values shown for each entry route are the experimentally determined fluxes (in nanomole/min-mg protein). Note that only 2% of the total influx is diffusional, 4% is associated with the serosal phloretin-sensitive system and the remainder (94%) is Na^+ -dependent and brush-border localized. These are mean values derived from experiments similar to those shown in Figures 1-3.

Experiments in which sugar uptake is followed for longer intervals show that the cells have a marked capacity to accumulate sugar against a concentration gradient as shown in Figure 5. Gradients of 10-15 fold are typically established when extracellular (3-OMG) is $100 \mu M$. The concentrative capability is entirely phlorizin-sensitive indicating that it is a function of the brush border Na^+ -dependent system. Detailed kinetic analysis of this system demonstrates that it has a K_T of approximately 1 mM and V_M of about 15 nmole/min-mg protein. Kinetic analysis of

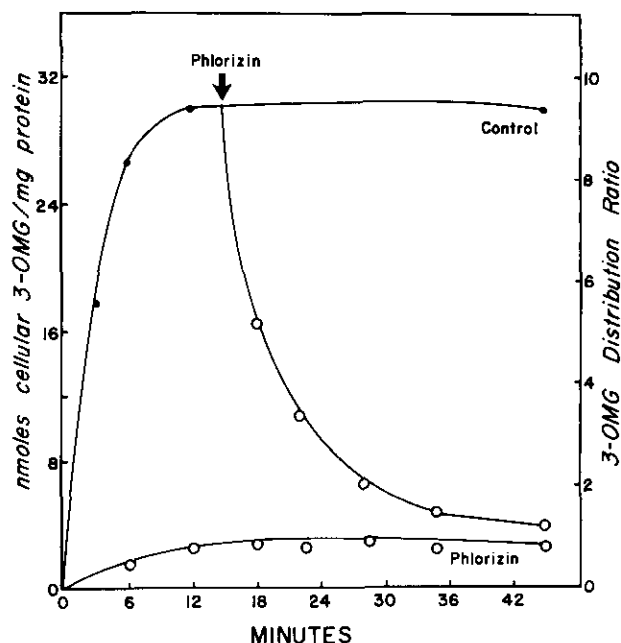


FIGURE 5. Accumulation of $100 \mu M$ 3-OMG by isolated enterocytes. The amount of 3-OMG taken up in the presence of phlorizin represents a steady state distribution ratio of 1.0. In the absence of phlorizin 3-OMG distribution ratios of 10-15 fold are typically observed. Phlorizin added after the steady state has been achieved induces a rapid release of accumulated sugar as shown.

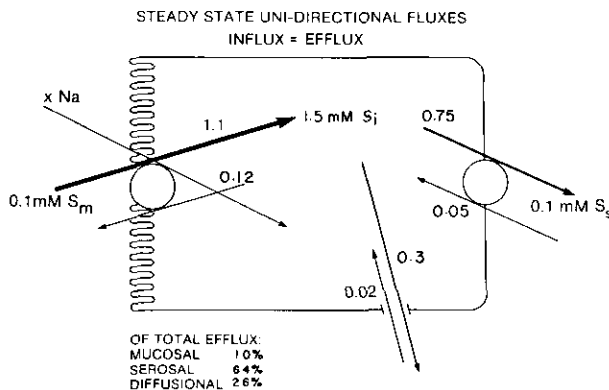


FIGURE 6. Schematic representation of steady state unidirectional 3-OMG fluxes in isolated intestinal epithelial cells. Values shown are in nmole/min-mg protein and have been calculated for a situation in which the cell is maintaining a 15-fold concentration gradient of sugar.

the Na^+ -independent phloretin-sensitive system indicate a K_T of about 75 mM and V_{max} near 40 nmole/min-mg protein (5, 12). The serosal carrier thus has a much higher capacity (V_{max}) than the mucosal carrier, and less tendency to saturate at high sugar concentrations (higher K_T). By making use of this kinetic

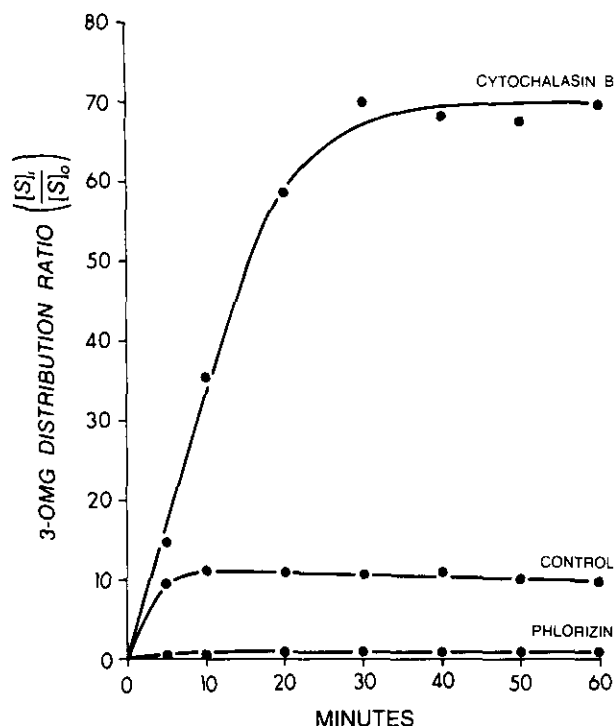


FIGURE 7. Effects of cytochalasin B (100 μM) or phlorizin (100 μM) on accumulation of 100 μM 3-OMG by isolated intestinal epithelial cells.

information, it is possible to calculate all of the steady-state unidirectional fluxes (influx and efflux) associated with each flux route. This has been done for a situation in which cells are maintaining a 15-fold concentration gradient (as often observed for an extracellular [3-OMG] of 100 μM), and the values are shown schematically in Figure 6. Note that at the steady state, 64% of the total sugar efflux from the cell occurs via the serosal carrier. Another 26% occurs via the diffusional route and only 10% of the total occurs by backflux on the Na^+ -dependent mucosal carrier. The system is well-designed to accomplish a rapid net transepithelial flow of sugar from mucosal to serosal boundaries as must occur physiologically.

On the other hand, presence of serosal and diffusional flux routes in the same cells as possess Na^+ -dependent transport capability causes significant problems in evaluating the energetics of Na^+ -dependent transport. The two passive systems seriously compromise the full gradient forming capability of the concentrative system. This can be demonstrated if the cells are incubated with any of the agents mentioned earlier that limit function of the serosal transport system. Under these conditions

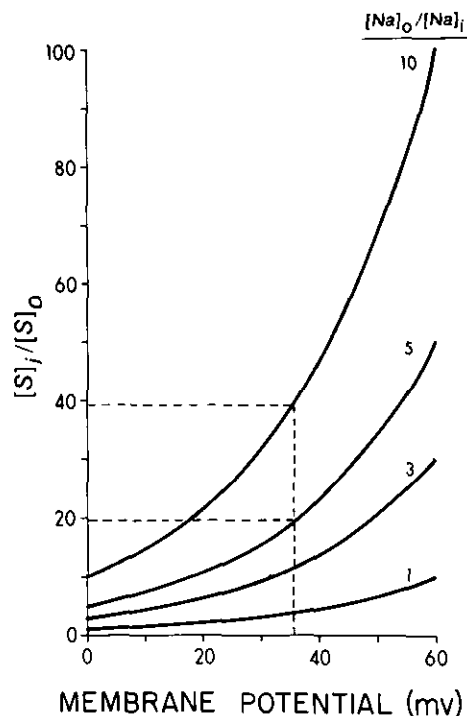


FIGURE 8. Relationship between expected maximum steady state sugar gradient and membrane potential for several different Na^+ gradients assuming a 1:1 Na:sugar coupling stoichiometry. From Kimmich, Carter-Su, and Randles (9).

they are able to establish considerably higher concentration gradients than when the efflux is not controlled (9). Optimal gradients of 70-fold are typically achieved when cytochalasin B is included as shown in Figure 7 (6).

Observed sugar gradients of 70 fold have raised significant questions regarding the energetics of Na^+ -dependent transport systems. As reviewed elsewhere (9, 13) these systems are thought to be driven by the transmembrane electrochemical potential for Na^+ . If all of the energy is from this source the relationship (1) must be obeyed:

$$RT \ln \frac{[S]_i}{[S]_o} \leq (RT \ln \frac{[Na]_o}{[Na]_i} + FV)n \quad (1)$$

Equation (1) simply says that the difference in chemical potential for sugar (between cell interior and medium) cannot be greater than the difference in electrochemical potential for Na^+ . The coefficient n is the number of Na^+ ions moving on the carrier per sugar molecule transferred on the same carrier. It has commonly been assumed to be unity based on experimental data obtained from Schultz's laboratory using rabbit intestine (4). By using this relation-

ship it is possible to construct the dependence between expected sugar gradients and the membrane potential for specific Na^+ gradients. A graph of this dependence is shown in Figure 8 for several different Na^+ gradients, and assuming that $n = 1$. Note that for a membrane potential of less than 60 mV, a 70-fold sugar gradient can only be achieved if the Na^+ gradient is at least 10-fold. In fact, the Na^+ gradient maintained by these cells is only about 5-fold, even assuming that the cellular activity coefficient for Na^+ is 0.5 (14). Furthermore, the mean value determined for the magnitude of the mucosal membrane potential for epithelial cells in intact tissue is -36 mV (15). It is unlikely that the isolated cells maintain any greater potential. For a Na^+ gradient of 5-fold and potential of 36 mV the maximum sugar gradient expected is about 20-fold. A membrane potential of 36 mV would only account for a 40-fold sugar gradient even if cellular Na^+ gradients are as high as 10-fold. Therefore, there is an apparent serious energy shortfall if one considers only the electrochemical gradient for Na^+ as the sole energy input for generation of sugar gradients by these cells. In addition, a

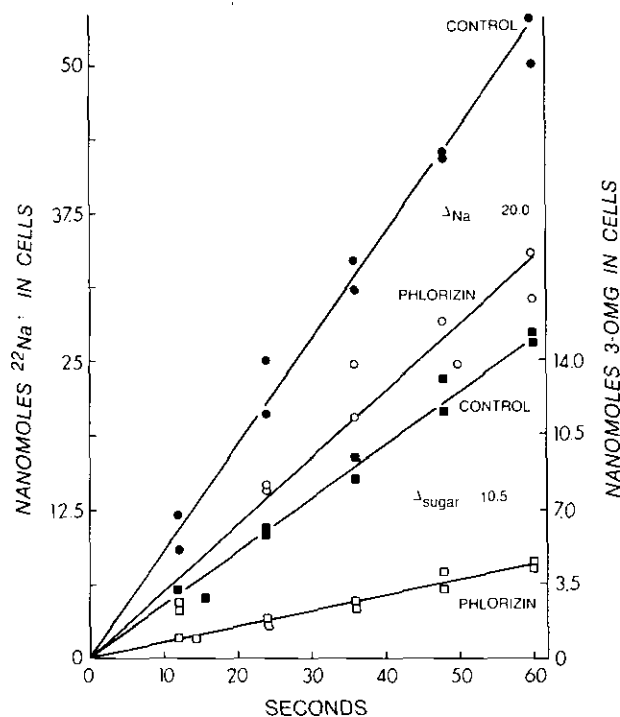


FIGURE 9. Effect of 200 μM phlorizin on unidirectional influx of Na^+ and 3-OMG in isolated enterocytes: (●, ■) control experiments; (○, □) obtained in the presence of phlorizin. Each experiment was run in the presence of 20 mM 3-OMG; Δ values indicate the difference in flux caused by phlorizin for each solute. The ratio of Δ values indicates a Na^+ :sugar coupling stoichiometry of 2.0.

70-fold sugar gradient still does not represent the optimal gradient expected. Recognize, that the diffusional flux pathway continues to act to limit the full

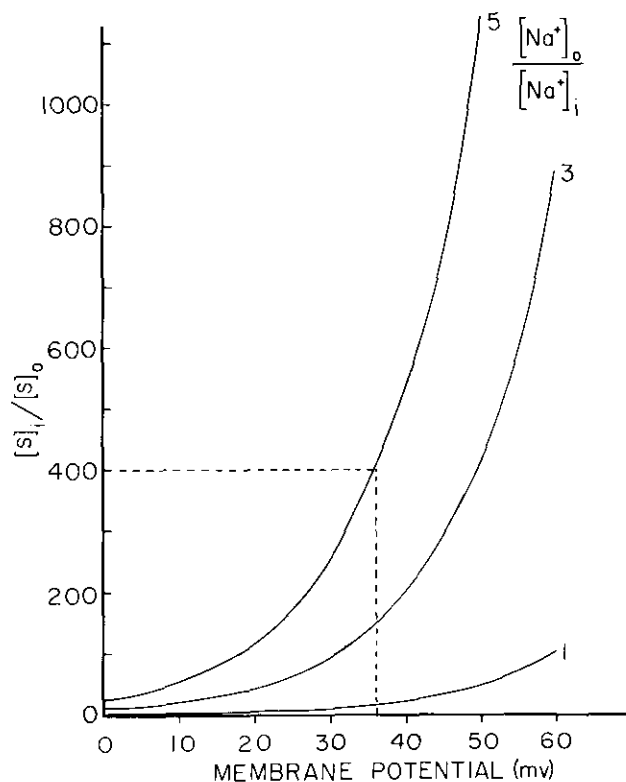


FIGURE 10. Relationship between expected maximum steady state sugar gradient and membrane potential for several different Na^+ gradients and assuming a 2:1 Na^+ :sugar coupling stoichiometry.

gradient forming capacity of the Na^+ -dependent system. In fact, kinetic analysis indicates that at the steady state with cytochalasin B present, more than 70% of the total efflux is now via the diffusional route. If this pathway could be blocked, then optimal sugar gradients much greater than 70-fold would be established. The true energy shortfall is even greater than it first appears.

Because of the information described above, and because there is no compelling evidence for an energy input to intestinal sugar transport systems beyond that represented by electrochemical Na^+ potentials, we have turned our attention to the possibility that the Na :sugar coupling stoichiometry (n) is greater than 1. In order to evaluate this possibility phlorizin-sensitive unidirectional fluxes of both Na^+ and sugar were determined. Cells were incubated with $^{22}\text{Na}^+$ (112.5 mM) and ^{14}C -3-OMG (20 mM),

either with or without 200 μM phlorizin. The difference in observed rates was determined in order to calculate a phlorizin-sensitive flux for each species and the ratio of these fluxes was taken as the coupling stoichiometry. A typical set of results is shown in Figure 9, which indicates that the value of n is equal to 2 rather than the formerly used value of 1. The total energy available from the electrochemical Na^+ gradient is thus considerably larger than usually recognized. The expected relationship between sugar gradients and membrane potential is sharply up-sweeping as shown in Figure 10. Note that in this case a membrane potential of 36 mV and sodium gradient of 5-fold is more than sufficient to account for experimentally observed sugar gradients of 70-fold. Indeed, gradients as high as 400-fold are theoretically possible. Given the fact that about 70% of the total sugar efflux is via diffusion when 70-fold sugar gradients are achieved, then a theoretical gradient of 400-fold in the absence of leaks does not seem unrealistic.

Conclusions

The isolated intestinal epithelial cell system has been extremely useful for exploring many of the details of intestinal sugar transport energetics as described above. It has proven particularly useful for examining epithelial flux pathways associated with serosal cell boundaries in a much more direct fashion than is possible for more conventional experimental systems employing intact tissue preparations. Because there are no significant extracellular compartments similar to those characteristic of intact tissue, kinetic analysis of steady state fluxes can be accomplished more directly and with a higher degree of confidence. By utilizing these advantages we have been able to: evaluate contributions of serosal transport systems to total epithelial cell sugar influx, evaluate the effect of agents which interact with serosal systems in terms of their effect on unidirectional sugar influx, maximize steady-state gradients maintained by the cells, evaluate current ideas regarding the sufficiency of the electrochemical potential for Na^+ as a driving force for Na^+ -dependent sugar transport, experimentally measure sugar-dependent unidirectional influx of Na^+ , and demonstrate that two Na^+ ions enter the epithelial cell per sugar molecule transported.

While we have emphasized use of the system for characterizing a specific transport system in terms of kinetics, capability and interaction with chemical agents, the same fundamental approach should prove useful for characterizing a variety of intestinal functional capabilities. In the case of sugars, a mucosal concentrative Na^+ -dependent pump in

combination with a high capacity serosal leak accounts for net epithelial solute transfer. A similar pump-leak process appears to operate for amino acids (16), phosphate (17), sulfate (18), bile salts (19, 20) and certain Krebs Cycle intermediates (21), although none of these systems have yet been characterized as thoroughly as that for sugars. Other solutes such as Na^+ and Ca^{2+} are transferred via a mucosal leak serosal pump system although again full characterization is lacking. Ultimately details of all of these capabilities will be established along with facts relating to the allosteric and hormonal regulation of each. Each element of the transport systems and their control systems can potentially be studied with the use of the isolated cell system as well as the manner in which a variety of environmental agents might act to alter their normal function. Because epithelial tissues represent the first site of interaction between ingested chemical agents and biological systems, the effects of such agents on epithelial cell function is of particular relevance. Work with intact tissue preparations is frequently difficult to interpret in light of the multiplicity of cell types present and uncertainties with regard to the tissue distribution of accumulated solute. The use of isolated epithelial cells circumvents both difficulties. Moreover, recent information from several laboratories (22-24) indicates that there is a very high degree of functional similarity between intestinal epithelium and proximal kidney tubule epithelium. It seems likely therefore that the intestinal cell system can serve as a model for function of kidney epithelium, and can be used to pinpoint possible renal effects of environmental agents which are much more difficult to study in kidney directly. The isolated intestinal cell system could prove to be a useful and timely scanning technique for detecting impairment of processes related to either intestinal or renal function. It may then be employed for the dual purpose of evaluating basic questions of cell function and for identifying biological sites of action of environmental agents.

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